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Microarray and cDNA sequence analysis of transcription during nerve-dependent limb regeneration.

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Public Summary:

BACKGROUND: Microarray analysis and 454 cDNA sequencing were used to investigate a centuries-old problem in regenerative biology: the basis of nerve-dependent limb regeneration in salamanders. Innervated (NR) and denervated (DL) forelimbs of Mexican axolotls were amputated and transcripts were sampled after 0, 5, and 14 days of regeneration. RESULTS: Considerable similarity was observed between NR and DL transcriptional programs at 5 and 14 days post amputation (dpa). Genes with extracellular functions that are critical to wound healing were upregulated while muscle-specific genes were downregulated. Thus, many processes that are regulated during early limb regeneration do not depend upon nerve-derived factors. The majority of the transcriptional differences between NR and DL limbs were correlated with blastema formation; cell numbers increased in NR limbs after 5 dpa and this yielded distinct transcriptional signatures of cell proliferation in NR limbs at 14 dpa. These transcriptional signatures were not observed in DL limbs. Instead, gene expression changes within DL limbs suggest more diverse and protracted wound-healing responses. 454 cDNA sequencing complemented the microarray analysis by providing deeper sampling of transcriptional programs and associated biological processes. Assembly of new 454 cDNA sequences with existing expressed sequence tag (EST) contigs from the Ambystoma EST database more than doubled (3935 to 9411) the number of non-redundant human-A. mexicanum orthologous sequences. CONCLUSION: Many new candidate gene sequences were discovered for the first time and these will greatly enable future studies of wound healing, epigenetics, genome stability, and nerve-dependent blastema formation and outgrowth using the axolotl model.

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